TO THE EDITOR

Human skin is colonized by a diverse array of microorganisms. The human skin microbiome is of great interest for its role in skin health and disease and as a potential target for therapeutic and cosmetic applications (Byrd et al., 2018). A major limitation in the laboratory investigation of microbial and host-microbe interactions on human skin is the lack of a model that can reliably reproduce the complexity of the host. Attempts to model human microbial communities in rodents are impeded by competition from native flora, and even germ-free models suffer because of the substantial differences between murine and human integumentary systems. Reconstructed human epidermis (RHE), epidermis generated from human stem cells and cultured on an air-liquid interface, lack the histologic, physiologic, and immunologic complexity of human skin. By contrast, skin explants from living human donors recapitulate with greater fidelity the skin’s physiology but may be subject to greater interdonor variability. We performed a pilot of microbiome and skin microbial colonization experiments with both RHE and explants and present our methods comparison here for the benefit of the community.

We sought to develop a standardized protocol in which defined microbial communities could be colonized onto a human skin model, intervened upon as needed, and subsequently analyzed. We tested three commercially available human skin models, all three cultured in an air-liquid interface. Epiderm (Mattek; Kubilus et al., 2004; Netzlaff et al., 2005) is an RHE differentiated from human foreskin—derived epidermal keratinocytes. In addition to a layer of epidermis, Labskin (Innoven; Harvey et al., 2016; Holland et al., 2008) adds a thick dermal matrix constructed from fibroblasts and is specifically advertised as a model for microbiome colonization. Finally, NativeSkin (Genoskin; Abadie et al., 2018; De Wever et al., 2015) are skin biopsies from living human donors that contain the full architecture of the human skin, including epidermis, dermis, skin appendages, and native immune cells (Figure 1a).

RHEs are differentiated in a sterile laboratory environment and can be treated as a germ-free model, accordingly. By contrast, explants from living donors, even when treated with anti-septics or antibiotics before surgery, may have a microbial background. To investigate this, we performed metagenomic whole genome shotgun sequencing and 16S rRNA amplicon
gene sequencing on explants from four different donors (Figure 1, Supplementary Figure S1, and Supplementary Table S1). We tested different microbiome collection methods (swabs, tissue dissociation, or whole-tissue homogenization) for facile investigation, particularly for shotgun metagenomics, whose sequencing depth can suffer significantly from human DNA admixture (Figure 1b−d). Dissociating and filtering samples yielded the highest number of unique species identified. However, whole-skin preparations, although comprehensive, result in exorbitantly high DNA admixture that limits recovery of microbial sequence reads. Although facile, swab-based methods were more dissimilar, potentially because swabbing has limited recovery of adherent microbes or those in secondary structures (e.g., biofilms) or appendages. Generally, we observed high relative abundances of Actinobacteria (Cutibacterium and Corynebacterium) and Firmicutes (Staphylococcus) common dominant skin microbes (Oh et al., 2014). As expected, donor-donor variability exceeded variation observed...
within a donor (Figure 1e), although for all donors, interestingly, microbiome composition remained largely stable over the explant life (Figure 1f and g). This, taken together with an inability to recover live microbes via cultivation at either timepoint (in contrast to RHEs, which were readily cultivatable; Supplementary Table S2), suggested that the pretreatments and/or subsequent tissue processing may have killed surface microbes, necessitating DNA-based methods to detect the variable donor background.

To compare how each system may be used for experimental skin microbiota modeling, we applied a mock bacterial skin community composed of four common skin commensals. After establishing each of the models as recommended by the manufacturer, we applied microbes and collected samples after 5–7 days to allow the community to equilibrate (Figure 2a). By colony forming unit analysis, qPCR, and 16S rRNA sequencing, we detected all predicted organisms on all models, with terminal composition varying by model and, in the case of explants, per donor (Figure 2b–d). Labskin yielded the most consistent results (Figure 2c, Supplementary Figure S2). We noted an expansion of staphylococci in all models and an expansion of the relative abundance of *Cutibacterium* only in the explant model. A potential explanation is that *Cutibacterium acnes* favors hypoxic, lipid-rich environments (Dreno et al., 2018), which are likely comparatively reduced or absent in RHE models. It is also possible that host-derived *Cutibacterium* could be expanding, although unlikely given the stability of no-microbe controls.

Finally, we compared 16S rRNA sequencing with qPCR, metagenomic whole genome shotgun sequencing, and colony quantitation (Supplementary Table S2) for assessing model utility. We observed consistent disparities between the microbial presence implied by nucleic acid analyses and the minimal or absent culturable organisms recovered. To explain this disparity, we applied a tool previously developed by our group to infer microbial growth rate from metagenomic whole genome shotgun sequencing data (Emiola and Oh, 2018). For the samples and organisms with adequate sequence depth to apply the growth rate index, growth rate indices indicative of active growth suggested a trend toward congruency with increased relative abundance (Figure 2b) and, to a lesser extent, the recovery of culturable organisms; however, this
analysis was limited because of the low microbial sequencing depth recoverable from explants. In our opinion, both RHE and explant models have advantages and disadvantages for skin microbiology assays. Our results suggest that RHE models may yield more reproducible results but may be less conducive to the study of aero-tolerant anaerobes like *C. acnes* than explant models. Explants more faithfully recapitulate the human epidermis, which may facilitate the study of a wider range of microbes and microbial communities and interactions with the skin’s innate immunity. However, residual antimicrobials from topical antibiotics and systemic presurgical prophylaxis may render the native microbiome of explant donors inert and interfere with experimentally applied organisms. Explants from non-prophylaxed donors that have been adequately cleaned before use may circumvent this issue. In addition, none of these models will be able to recapitulate human diversity that can affect microbiome characteristics, such as ethnicity, sex, age, and genetic diversity; ex vivo models are skewed toward demographics that have higher elective cosmetic surgeries, and RHEs are derived in batches from different foreskin donors. Finally, when applying high concentrations of bacteria to a small model, nucleic acid amplification assays are particularly prone to over-representing nonculturable and potentially dead organisms. Culture-based assays or high-depth metagenomic whole genome shotgun sequencing allow greater confidence in the results of ex vivo skin community models.

Additional limitations common among these models include their short lifespan, which may limit studies with slower-growing microbes, biofilms, or those examining microbiome stability; lack of secondary structures (RHEs); and the relatively high cost per unit. Despite these caveats, we believe that all of these models can be useful for studying short-term microbiome and host-microbiome interactions and for safety testing and pathogen or probiotic intervention studies. In addition, we expect that they can be used to investigate important knowledge gaps in microbiome dysbiosis. For example, mock communities could be colonized onto skin models and intervened upon to cause or resolve dysbiosis. It is also possible that patient samples could, to a degree, be transferred to a model, but we expect there to be major technical considerations requiring optimization, including accurate representation of microbial load, diversity, live-dead, and presence in secondary structure. Furthermore, although not explored in this paper, fibroblast (Labskin) and resident leukocyte immune activation (NativeSkin) could be measured in response to model dysbioses. We hope this letter assists other researchers in their implementation of laboratory skin microbiome experiments.

**Data availability statement**

Datasets related to this article can be requested from the authors upon request.

**Ethics Statement**

The Jackson Laboratory Institutional Review Board determined that this project does not meet the definition of human subjects research under laboratory policy and applicable federal regulations.

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**CONFLICT OF INTEREST**

The authors state no financial conflict of interest.

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**AUTHOR CONTRIBUTIONS**

Conceptualization: JQ, PJL, DC, EF; Data Curation: PJL, JO, DC; Formal Analysis: PJL, DC; Funding Acquisition: JQ; Investigation: PJL, DC, EF; Methodology: PJL, EF, DC; Project Administration: JQ; Resources: EF, JQ, PJL; Software: PJL, JO, DC; Validation: PJL, EF; DC; Visualization: PJL, DC; Writing - Original Draft Preparation: PJL; Writing - Review and Editing: JQ, PJL

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**SUPPLEMENTARY MATERIAL**

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2020.05.096.

**REFERENCES**


De Wever B, Kundykowski S, Descargues P. Human skin models for research applications in pharmacology and toxicology: introducing NativeSkin®, the “missing link” bridging cell culture and/or reconstructed skin models and human clinical testing. Applied In Vitro Toxicology 2015;1:26–32.


**SUPPLEMENTARY MATERIALS AND METHODS**

**Mock community assembly and application**

*Staphylococcus epidermidis* (ATCC 12228), *Micrococcus luteus* (ATCC 4698), *Corynebacterium pseudogenitalium* (ATCC 33035), and *Cutibacterium acnes* (ATCC 6919) were obtained from ATCC and cultured before inoculation onto skin models according to ATCC guidelines. The *Staphylococcus capitis* strain was isolated previously by our group. *S. epidermidis, S. capitis*, and *M. luteus* were grown up in tryptic soy broth at 37 °C and 220 revolutions per minute. *C. pseudogenitalium* was grown up in tryptic soy broth +1% Tween at 37 °C and 220 revolutions per minute. *C. acnes* was grown up in tryptic soy broth anaerobically at 37 °C. To control the proportions of each taxa added to mock communities, we performed growth curves to estimate the colony forming units/ml at optical density 660 0.8. Cultures were grown to optical density 0.8, pelleted by centrifugation at 4,696 relative centrifugal force for 10 minutes, and resuspended in 0.1 mm zirconia beads (BioSpec Products). Suspensions of different species were then combined in a ratio to yield a representative skin community, then combined with an equal volume of 40% glycerol for long-term storage for uniformity of inoculum between batches. We tried ratios of *Staphylococcus*/*Corynebacterium/Cutibacterium/Micrococcus* 1:1:1:1, 36:1:1:1, and 1:1:36:1. The mock community featured in the results was 36:1:1:1, which ultimately yielded a composition that most resembled dry skin communities according to our published data.

Mock communities were applied to skin models by pipetting 10 μl directly to the surface of the center of the model to prevent contamination of the surrounding media. We tested spreading the solution around with a sterile glass stir rod but found that this risked compromising the integrity of the membrane of the Epiderm models, allowing bacteria to contaminate the media, so we do not recommend this practice.

**Bacterial quantitation**

For identification of cultivable organisms recovered from skin models, samples were serially diluted and plated onto both TSA and Bordet Gengou Blood Agar (Thermo Fisher Scientific, Waltham, MA) plates, then incubated aerobically and anaerobically at 37 °C for 72 hours or until visible colonies grew. Colonies were classified using a MALDI Biotyper system (Bruker Corporation, Billerica, MA) according to manufacturer protocols.

**Human skin model preparation and culture**

Reconstructed human epidermis models were obtained from Matttek (Epiderm) and Innoven (Labskin), and human skin explants were obtained from Genoskin. The Jackson Laboratory Institutional Review Board determined that this project does not meet the definition of human subjects research under laboratory policy and applicable federal regulations.

All models were revived and maintained in an air-liquid interface using manufacturer supplied antibiotic-free media in 37 °C with 5% CO2, according to manufacturer protocols.

**Sample DNA extraction**

We tried several methods for recovering microbial communities from skin models.

**Dissociation and filtration.** Skin models were harvested for metagenomic extraction by first dissecting them from their manufacturer membrane or agar matrix with sterile surgical instruments, cutting them in half and placing each half into an autoclaved 1.7 ml Eppendorf nuclease-free tube containing either 0.5 ml PBS for bacterial cultivation or 0.3 ml of sterile Tissue & Cell Lysis Solution (Lucigen, Middleton, WI) for nucleic acid analysis. Samples were then dissociated using a Tissue Dissociator (Thermo Fisher Scientific) with a sterile pestle at maximum power for 10 seconds. Next, samples were filtered through a 40-μm cell strainer (Thermo Fisher Scientific) into a 50-ml conical tube (Eppendorf, Hauppauge, NY), then transferred to a sterile, nuclease-free 2.0-ml Eppendorf Safe-Lock tube containing 100 μg of autoclaved 0.1 mm zirconia beads (BioSpec Products, Bartlesville, OK).

**Surface swab.** Skin swabs were collected using a PurFlock Ultra buccal swabs (Puritan, Guilford, ME) that had been dipped into sterile PBS to swab the area. The swab was placed into a microfuge tube containing 350 μl of Tissue and Cell lysis buffer (Epicentre, Madison, WI) and 100 μg of 0.1-mm zirconia beads (BioSpec Products).

**Whole-skin processing.** Skin models were dissected them from their agar matrices and transferred to a sterile, nuclease-free 2.0-ml Eppendorf Safe-Lock tube containing 100 μg of autoclaved 0.1 mm zirconia beads (BioSpec Products) and either 0.5 ml PBS for bacterial cultivation or 0.3 ml of sterile Tissue & Cell Lysis Solution (Lucigen) for nucleic acid analysis.

**Metagenomic DNA extraction**

Metagenomic DNA was extracted using the GenElute Bacterial DNA Isolation kit (Millipore Sigma, Burlington, MA) according to manufacturer protocol with the following modifications standard for our lab: each sample digested with 50 μg of lysozyme, 5 units lysostaphin, and 5 units mutanolysin for 30 minutes before bead-beating in the Tissuelyser II (Qiagen, Hilden, Germany) for 2 × 3 minutes at 30 Hz. Samples were centrifuged for 1 minute at 15,000 g before loading onto the GenElute column. Negative (environmental) controls and positive (mock community) controls were extracted and sequenced with each extraction and library preparation batch to ensure sample integrity.

**QPCR quantitation of bacterial abundance**

To estimate the abundance of bacteria in a sample from isolated DNA, 100—175 base pair QPCR primers were designed for each strain using NCBI Primer BLAST of species-specific genes identified with Chocophlan. PCR cycling conditions using PowerUp SYBR Green Master Mix (Thermo Fisher Scientific) were optimized on a ViiA 7 Real-Time PCR System (Thermo Fisher Scientific). Primers were validated against genomic DNA isolated from each strain using a GenElute Bacterial DNA Isolation kit (Millipore Sigma). Colony forming units/CT standard curves were conducted for each species by growing cultures grown to optical density 660 nm 0.8, then quantitating cultures by both serial dilution onto agar plates, DNA isolation, and QPCR.

**16S rRNA gene amplicon sequencing**

16S V1-V3 PCR amplification, library prep, and sequencing were performed...
by the Jackson Laboratory for Genomic Medicine Microbial Genomic Services and Genomic Technologies cores as previously described (Svenson et al., 2019).

**Metagenomic whole genome shotgun sequencing**

DNA sample concentrations were determined by Qubit HS (Thermo Fisher Scientific) and diluted to 1 ng/μl. Metagenomic whole genome shotgun sequencing libraries were made according to the optimized quarter reaction Illumina protocol using the Nextera XT kit (Illumina, Thermo Fisher Scientific), where all reagents for library preparation were taken in one quarter of the amount. The dual indexed paired-end libraries of genomic DNA were made with an average insert size of 400 base pairs by taking 200 pg DNA of each sample. Tagmentation and PCR reactions were carried out according to the manufacturer’s instructions. Resulting Nextera whole genome sequencing libraries were sequenced on an Illumina HiSeq2500 with 2/×2 150 base pairs paired-end reads to a sequencing depth up to 240 million reads/sample.

**Bioinformatics**

**16S rRNA gene amplicon data.** 16S rRNA gene amplicon reads were prepared for analysis using VSEARCH v2.6.2 (Rognes et al., 2016). Demultiplexed Illumina reads were first combined into a single file and merged using -fastq_mergepairs. Merged reads were then filtered using -fastq_filter, requiring a minimum length of 250 base pairs, maximum consecutive Ns of 8, and maximum expected errors of 1. Reads passing quality control were then dereplicated with removal of unique reads with less than five representations in the dataset. Chimeric sequences were detected and removed using -uchime_denovo. Sequences were clustered into operational taxonomic units using -cluster_fast with a 97% identity cutoff. Original reads were then mapped to the operational taxonomic units using -usearch_global to create an operational taxonomic unit table. Operational taxonomic units were classified using the -sintax command in USEARCH v 8.0.1517 (Edgar, 2010), aligning to the Silva silva_16s_v123 database (Glöckner et al., 2017).

**Statistics and significance test**

Two-group nonparametric bidirectional comparisons were conducted using the Wilcoxon rank sum test with continuity correction in R using the wilcox.test() command. One way ANOVA on ranks was conducted using the Kruskal-Wallis H test kruskal.test() command. α = 0.05 for all applicable statistical tests conducted in this study.

**SUPPLEMENTARY REFERENCES**


Supplementary Figure S1. Inter-donor and intra-donor metagenomic background heterogeneity. (a) Comparison of microbiome collection methods by individual sample from donor 4. Barplots show relative abundance of a donor microbiome obtained via tissue dissociation and filtration, whole-skin bead-beating, or surface sampling with a flocked swab. X-axis labels indicate day, replicate, and collection method. (b) NativeSkin donor background by individual sample. Relative abundance of endogenous NativeSkin flora by donor. Each bar represents a single explant. 16S rRNA gene amplicon analysis, plotting the 15 most abundant genera. X-axis labels indicate day of collection and replicate. Two of day 7 samples were omitted because of low read count.

Supplementary Figure S2. Mock community culture on human skin models experiments by individual sample, indicated by “_#”. Relative abundance of 16S rRNA gene amplicon analysis, plotting the 15 most abundant species. Each bar represents a single explant. X-axis labels indicate model and replicate. Two NativeSkin samples are omitted because of low read count.
### Supplementary Table S1. Demographics of Donors of NativeSkin (Genoskin) Explants

<table>
<thead>
<tr>
<th>Donor</th>
<th>Lot</th>
<th>Sex</th>
<th>Age</th>
<th>Ethnicity</th>
<th>Fitzpatrick scale</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>QUO126/20190213.1</td>
<td>F</td>
<td>47</td>
<td>Not Hispanic or Latino</td>
<td>2</td>
<td>abdomen</td>
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<tr>
<td>2</td>
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<td>NP</td>
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<td>abdomen</td>
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</tbody>
</table>

Abbreviations: F, female; NP, not provided.

### Supplementary Table S2. Comparative Results for Quantifying Presence of Bacteria

<table>
<thead>
<tr>
<th>Model</th>
<th>CFUs Recovered</th>
<th>qPCR Predicted CFUs</th>
<th>GRID</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Staphylococcal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epiderm</td>
<td>1.7E7 (2.6E7)</td>
<td>6.5E10 (6.2E10)</td>
<td>ND</td>
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<tr>
<td>Labskin</td>
<td>7.1E5 (6.9E5)</td>
<td>ND</td>
<td>2.31</td>
</tr>
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<td>NativeSkin</td>
<td>TNTC</td>
<td>ND</td>
<td>1.5</td>
</tr>
<tr>
<td>Inoculum</td>
<td>3.4E4</td>
<td>4.1E5</td>
<td>1.49</td>
</tr>
<tr>
<td><strong>Cutibacterial</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epiderm</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Labskin</td>
<td>—</td>
<td>ND</td>
<td>ID</td>
</tr>
<tr>
<td>NativeSkin</td>
<td>—</td>
<td>ND</td>
<td>1.14</td>
</tr>
<tr>
<td>Inoculum</td>
<td>2.0E3</td>
<td>4.7E3</td>
<td>1.13</td>
</tr>
<tr>
<td><strong>Corynebacterial</strong></td>
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<td></td>
<td></td>
</tr>
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<td>Epiderm</td>
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<td>ND</td>
</tr>
<tr>
<td>Labskin</td>
<td>—</td>
<td>ND</td>
<td>ID</td>
</tr>
<tr>
<td>NativeSkin</td>
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<td>ND</td>
<td>ID</td>
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<tr>
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<td><strong>Micrococcal</strong></td>
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</tr>
<tr>
<td>Labskin</td>
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<td>ND</td>
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</tr>
<tr>
<td>NativeSkin</td>
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<tr>
<td>Inoculum</td>
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</tr>
</tbody>
</table>

Abbreviations: CFU, colony forming unit; GRID, growth rate index; ID, insufficient depth; mWGS, metagenomic whole genome shotgun sequencing; ND, not done; TNTC, too numerous to count.

Each model was inoculated with a mock community in replicate (Epiderm [n = 4], Labskin [n = 3], and NativeSkin [n = 2]), and at the end of the model life, microbial taxa were quantified with a combination of culture- and nucleic acid–based methods. Number indicates mean value, number in parenthesis indicates standard deviation.

1Only one sample yielded sufficient mWGS depth of coverage to calculate GRID.